

Supplementary Table 1: Gene description and List of primers used in the study

Primer Name	Gene number and Gene name	Gene description	Gene essentiality *	Sequence	Size of primer (bp)
Ndel_rib A2_F	<i>Rv1415</i> (MT1458) <i>ribA2</i>	bifunctional riboflavin biosynthesis GTP cyclohydrolase II/3,4-dihydroxy-2-butanone 4-phosphate synthase	Essential	GGGGCATATGATGAC GAGGTTGGACTCCGT	30
MluI_rib A2_R				GGGGACGCCTCAC AAGGCACCGCCGAAT TCTC	33
Ndel_rib F_F	<i>Rv2786c</i> (MT2856) <i>ribF</i>	bifunctional riboflavin kinase/FMN adenylyltransferase	Essential	GGGGCATATGGTGC GCCGTAGGCTTGCCT TCGT	33
MluI_rib F_R				GGGGACGCCTCAA CCCGTAGACAACAGG TCAC	33
Ndel_rib G_F	<i>Rv1409</i> (MT1453) <i>ribG</i>	bifunctional riboflavin biosynthesis diaminohydroxyphospho ribosylaminopyrimidine deaminase/5-amino-6-(5-phosphoribosylamino) uracil reductase	Essential	GGGGCATATGATGAA CGTGGAGCAGGTCAA GAG	33
PstI_rib G_R				GGGGCTGCAGCTAA CGAGCCACCAAGCTC AGCA	33
Ndel_rib H_F	<i>Rv1416</i> (MT1459) <i>ribH</i>	6,7-dimethyl-8-ribityllumazine synthase	Essential	GGGGCATATGGTGAA GGGTGGCGCCGGGG TGCC	33
MluI_rib H_R				GGGGACGCCTCAC GAGTGAGCGCGCAG CTCGC	33
Kan(F)	Kan ^r	Kanamycin resistance gene		GAGAAAACTCACCGA GGCAG	20
Kan(R)				GTATTTCGTCTCGCT CAGGC	20

* Gene numbers; Rv- notation is used to reflect gene number in *M. tuberculosis* H37Rv; MT- notation is used to reflect gene number in *M. tuberculosis* CDC1551. However, at sequence level riboflavin biosynthetic genes (ribA2, ribF, ribG and ribH) of H37Rv bear 100% identity to respective genes in CDC1551. The riboflavin biosynthetic pathway is represented as per the information available in kegg pathway (https://www.genome.jp/kegg-bin/show_pathway?mtu00740 for *M. tuberculosis* H37Rv and <https://www.genome.jp/pathway/mtc00740> for *M. tuberculosis* CDC1551)

** Gene essentiality information is based on <https://webhost.ntu.edu/target/Default.aspx>. Bosch, B et al, 2021; DeJesus, MA et al, 2017.